ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Evaluation of Some Biocontrol Agents against Soil Pathogenic Fungi.

Abou-Aly HE [1], Neweigy NA[1], Zaghloul RA[1], El-Sayed SA[2] and Bahloul AM[1]

[1]Fac. Agric., Moshtohor, Benha University
[2]Soils, Water and Environment Research Inst. Agric. Research Center.

ABSTRACT

The current study was aimed to screen of certain rhizospheric bacterial biocontrol agents against some pathogenic fungi. The obtained data revealed that among 116 bacterial isolates, nineteen bacterial isolates showed the highest inhibition percentage against *Fusarium oxysporum, Rhizoctonia solani* and *Sclerotium rolfsii*. Regarding the antifungal activities, results indicated that isolates B38 and B103 recorded the higher values of chitinase activity, catecholate-type siderophores and NH_3. Also data showed that isolate B103 was the highest producer for HCN followed by the isolates number B13 and B38. Concerning the production of volatile compounds, data indicated that isolates B103 and B38 showed considerable inhibition against pathogenic fungal growth. Data also showed that poly medium was the best medium for production of inhibitive substances for pathogenic fungi by the tested bacteria. Moreover, cultural filtrates of bacterial biocontrol agents with concentration of 10% showed the highest reduction percentage of mycelial weight of the tested strains after 3 days. The most potent isolates for bioagent production were chosen and these isolates were identified as *Pseudomonas fluorescens* (B103) and *Bacillus subtilis* (B38).

Keywords: biocontrol agents, *F. oxysporum, R. solani, S. rolfsii*, chitinase, siderophores, NH_3, HCN, volatile antibiotics, *Pseudomonas fluorescens*, *Bacillus subtilis*.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Bacillus species are widely used in the biocontrol of plant diseases for more than 50 years, because they have a well developed secretary system producing structurally diverse secondary metabolites with a wide spectrum of antagonistic activity (Liu *et al*, 2007). Fluorescent pseudomonads represent an important group of rhizospheric bacteria that have promising antagonistic potential for use in biological control of soil-borne fungal pathogens because of their catabolic versatility, their excellent root-colonizing abilities and their capacity to produce a wide range of antifungal metabolites (De La Fuente *et al*, 2004).

Identifying the different mechanisms of biocontrol is important because it may provide a means of attacking pathogens with a broader spectrum of microbial weapons. Weller and Tomashow (1993) found that *Ps. fluorescens* produced several bioactive compounds (antibiotics, siderophores, HCN, indole acetic acid and volatile compounds) giving one of the broadest spectra of potential biocontrol. Chitinase could play an important role in the control of pathogenic fungi or enhancing plant disease tolerance. *Bacillus subtilis* is well known to be an abundant extra-cellular enzymes producer, including cellulase, glucanase, amylase, proteinase and chitinase. The ability to lyse fungal hyphae presents the possibility that living fungal hyphae, rather than chitin, may form the actual growth substrate for chitinolytic soil bacteria (Schallmey *et al*, 2004).

Siderophores are iron-chelating molecules produced by microorganisms in response to low environmental iron concentrations. These molecules help the microorganism to acquire iron, which is often biologically unavailable in the environment, and create iron-limiting conditions for competitors, including pathogenic microorganisms (Meyer and Stintzi, 1998). Many workers reported that the production of volatile ammonia by *Pseudomonas* spp. has been indicated as a possible mechanism to control soil borne pathogens (Baligh *et al*, 1996). Albuquerque *et al* (2003) and Zou *et al* (2007) reported that the production of HCN and volatile compounds showed antibiosis against soil borne pathogenic fungi. Finally, the search for antagonistic compounds in cultural filtrates has greatly intensified in recent years, due to the number of toxins, enzymes or compounds derivable from their metabolites.

The main objective of this study was concerned with the evaluation of bacterial isolated from local soils for controlling of some pathogenic fungi *in vitro*. Antagonistic tools (excretion of lytic enzymes, siderophoric compounds, ammonia, hydrogen cyanide and volatile antibiotics) were also studied.

MATERIALS AND METHODS

Isolation of biocontrol agent

Isolation of different bacterial isolates was carried out on different specific bacteriological media namely nutrient agar medium, tryptic soya agar medium, colloidal chitin agar medium and King's agar medium (Atlas, 2005) from rhizosphere of tomato, cucumber, peppers, beans, mallow, cabbage and eggplant.

Isolation and identification of pathogenic fungi

Isolation of pathogenic fungi was carried out on potato dextrose agar medium (Ricker and Ricker, 1936). One fungal strain of *Sclerotium rolfsii* was kindly obtained from Plant Pathology Research Institute, Agricultural Research Center, Ministry of Agriculture, Giza, Egypt. The key of the genus *Fusarium* reported by Nelson *et al* (1983) was followed in this respect. The other fungal genera were identified according to Barnett and Hunter (1987).

Screening of bacterial isolates

Bacterial isolates were screened on nutrient agar medium for their potentialities to inhibit the pathogenic fungal growth using dual culture technique as described by Hariprasad and Niranjana (2008).

Determination of bioactive agents by bacterial isolates

Bacterial isolates which showed considerable inhibition percentage for the tested pathogenic fungi were investigated for their potentialities for chitinase, siderophores, ammonia, hydrogen cyanide and volatile

antibiotics secretion according to the methods of Monreal and Reese (1969), Arnow (1937), Cappuccino and Sherman (1992), Lorck (1948) and Montealegre *et al* (2003), respectively.

Identification of the potent isolates

The potent bacterial isolates which showed potentialities of bioactive agents were identified according to Reva *et al* (2001) and Palleroni (2005).

Effect of cultivation media on production of bioagent by the potent strains

Identified bacterial strains were used to produce bioactive agents on five microbiological media namely nutrient medium as a control, tryptic soy medium, poly medium (Bourgouin *et al*., 1984), Emerson's medium (Weiss, 1975) and acetate yeast extract medium (Sasaki *et al*., 1998).

Effect of bacterial cultural filtrate on reduction of fungal biomass

Identified bacterial strains were grown on the best medium using shaking incubator (200 rpm) for 3 days at 30˚ C. 10%, 5% and 2.5% of each bacterial culture were separately added in 44 ml potato dextrose broth medium in conical flask, flasks containing the medium without bacterial filtrates but containing 6 ml sterilized distilled water was used as a control. All flasks were inoculated with pathogenic fungi and incubated at 28˚C. After 3 and 7 days, the fungal mats were harvested and mycelial dry weight was determined. The inhibition percentage in mycelial growth of the pathogenic fungi was determined according to the following equation: Reduction (%) = control - treatment / control × 100

RESULTS AND DISCUSSION

Isolation of biocontrol agents and pathogenic fungi

One hundred and sixteen bacterial isolates were obtained from different plant rhizosphere. Two pathogenic fungal isolates namely *Fusarium* sp. and *Rhizoctonia* sp. were obtained from different naturally infected plant and identified as *Fusarium oxysporum* and *Rhizoctonia solani*.

Screening of bacterial isolates

Screening was depended upon the ability of the obtained bacterial isolates to inhibit the growth of soil-borne pathogenic fungi. The obtained data illustrated in Figs 1, 2 and 3, showed that most of the tested bacterial isolates were varied in their inhibition for the growth of *F. oxysporum, R. solani* and *S. rolfsii.* About 96.6% of the tested bacterial isolates showed various inhibition effects against *F. oxysporum*.

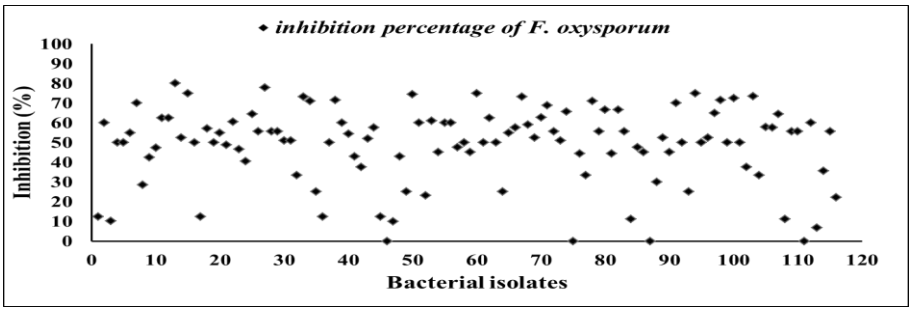

Fig 1. Inhibition percentage of *F. oxysporum* as affected by bacterial isolates

Isolate B13 was the most efficient for inhibiting the growth of pathogenic fungus by 80%, while, isolate B113 was the lowest one (6.7%). Concerning the *R. solani*, data illustrated in Fig 2 showed that 93.1% of the tested bacterial isolates exhibited inhibition activities against the growth of *R. solani*. Isolate B38 showed the highest inhibition activity (77.4%), while, isolate B32 was the lowest one to be 2.2%. Obtained results illustrated in Fig 3 clearly indicated that, the inhibition of *S. rolfsii* was obtained by 84.5% of the tested

bacteria. The most efficient inhibition was obtained by isolate B101 (82.2%), while, only 2.4% inhibition was found by isolate B48.

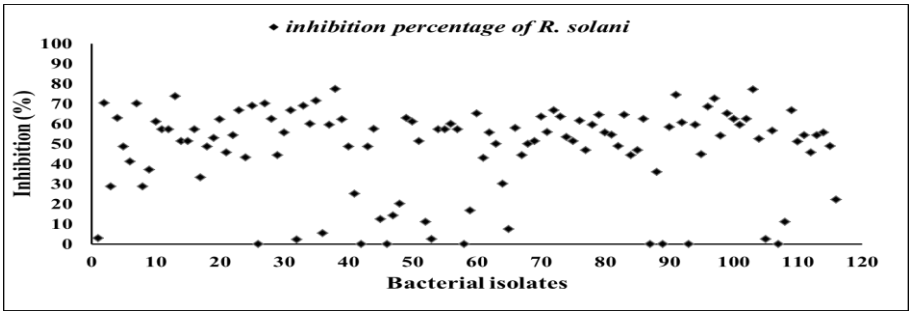

Fig 2. Inhibition percentage of *R. solani* as affected by bacterial isolates

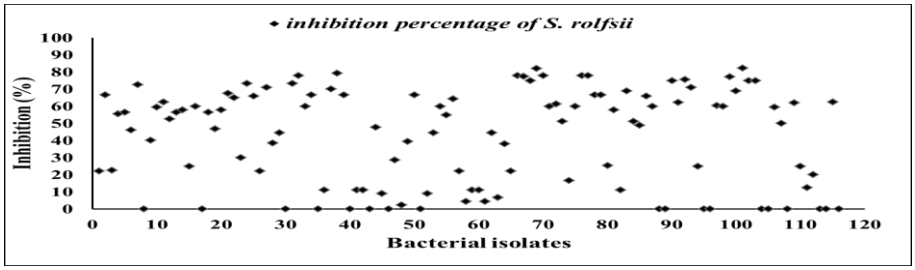

Fig 3. Inhibition percentage of *S. rolfsii* as affected by bacterial isolates

The inhibition of pathogenic fungi by the isolated bacteria could be attributed to the antifungal metabolites production such as, ammonia, siderophores, cell wall degrading enzyme; cellulase, chitinase and proteolytic enzyme (Chaiharn *et al*, 2008). In addition, other microbial by-products may also play an important role for inhibition of pathogenic fungi, for example, hydrogen cyanide (HCN) and volatile antibiotics (Phillips *et al*, 2004).

Determination of bioactive agents by bacterial isolates Chitinase, siderophores and ammonia production

Data in Table 1 indicated that all the selected bacteria have the ability to produce chitinase. Isolates B38 and B103 showed higher chitinase activities. While, isolates B02 and B13 showed the lowest chitinase activities. Microbial chitinolytic enzymes have been considered important in the biological control of many plant pathogens because of their ability to degrade fungal cell walls. Lytic enzymes are among these metabolites which can break-down polymeric compounds, including chitin, proteins, cellulose, hemicellulose (Ramyasmruthi *et al*, 2012).

On the other hand, all the selected bacterial isolates exhibited the ability to produce ammonia. The higher amounts of ammonia were detected by B38, B78 and B103 bacterial isolates. These results are in agreement with those obtained by Chaiharn *et al* (2008) who screened some rhizobacteria for production of antifungal metabolites such as ammonia. They found that more than 64% of the isolates produced ammonia.

Hydrogen cyanide and volatile antibiotics production

Data presented in Table 2 revealed that 84.2% of the selected bacteria were able to produce HCN in culture media, whereas 15.8% of them gave negative results. Also, the isolate number B103 was the highest HCN producer followed by the isolates numbers B13, B38 and B78.

Cyanide can be a ligand of hydrogenase in certain aerobic bacteria which are not described as cyanogenic bacteria (Blumer and Haas, 2000). In this respect, Weller and Tomashow (1993) reported that *Ps. fluorescens* produces several bioactive compounds (antibiotics, siderophores, HCN, indole acetic acid and volatile compounds) giving one of the broadest spectra of potential biocontrol.

ISSN: 0975-8585

Table 1. Determination of chitinase activity, catecholate-type siderophores and ammonia (NH_3) produced by the selected bacterial isolates

Isolates number	Chitinase activity (mM NAG/ 1ml /1 hr)	Catecholate-type siderophores detection	NH_3 production	Isolates number	Chitinase activity (mM NAG/ 1ml /1 hr)	Catecholate-type siderophores detection	NH_3 production
B02	1.91	+	+	**B70**	2.96	++	++
B07	2.73	+	++	**B78**	4.44	+	+++
B13	1.90	+	++	**B91**	3.02	-	+
B27	2.06	++	++	**B92**	2.49	+	+
B31	1.92	++	++	**B97**	2.49	+	+
B33	2.60	++	++	**B99**	4.39	+	+
B38	7.48	+++	+++	**B100**	4.60	+	++
B39	3.40	+	+	**B101**	2.09	+	+
B50	1.94	-	++	**B103**	5.88	++++	+++
B69	4.42	++	++	-	-	-	-

Table 2. HCN detection for the selected bacterial isolates and effect of volatile antibiotics on radial growth of pathogenic fungi

Isolates number	HCN detection	Inhibition by volatile antibiotics (%)			Isolates number	HCN detection	Inhibition by volatile antibiotics (%)		
		F	R	S			F	R	S
B02	+	30.0	66.7	36.6	**B70**	++	56.7	66.7	63.8
B07	+	13.3	49.0	43.0	**B78**	+++	49.5	71.2	74.2
B13	+++	45.0	71.8	51.7	**B91**	+	28.3	59.1	29.0
B27	-	21.7	18.8	00.0	**B92**	+	39.3	63.3	55.5
B31	+	51.7	67.8	61.8	**B97**	++	48.7	69.8	42.6
B33	+	50.0	66.7	57.6	**B99**	+	53.3	56.7	55.0
B38	+++	58.3	73.2	74.0	**B100**	-	25.0	24.8	18.1
B39	+	41.7	63.3	60.0	**B101**	+	50.0	62.2	33.3
B50	+	41.7	11.1	61.6	**B103**	+++	61.6	76.7	75.4
B69	-	31.6	16.7	27.8	**-**	-	-	-	-

- **F:** ***F. oxysporum*****, R:** ***R. solani*****, S:** ***S. rolfsii***

Moreover, the isolates B103 and B38 showed high inhibitory effect on *F. oxysporum* growth with a percentage of 61.6% and 58.3%, respectively. Regarding the *R. solani*, data showed that the isolate B103 was more efficient to inhibit the radial growth of the tested fungi (76.7%) followed by isolate B38 (73.2%). Concerning the *S. rolfsii,* the efficient inhibition of radial growth was obtained by the isolate B103 (75.4%) followed by B78 (74.2%). Similar results were obtained by Mojica *et al* (2008) who screened 6 strains of *Bacillus thuringiensis* against *Rhizoctonia solani* to produce volatile antibiotics. They reported that all the tested strains showed inhibitory effect in comparison with the control. *B. thuringiensis* strains GM-11 and GM-121 showed the best inhibitory effect on *R. solani* to be 76.88% and 74.66%, respectively.

Identification of the potent bioagent isolates

From the morphological characteristics, staining properties, spore formation and physiological properties according to Reva *et al* (2001) and Palleroni (2005), it was clear that the two isolates could be identified as *Pseudomonas fluorescens* B103 and *Bacillus subtilis* B38.

ISSN: 0975-8585

Effect of cultivation media on antifungal agents production

Data in Tables 3a, b and 4a, b show that, the effect of cultivation media on antifungal agents production i.e. volatile antibiotics, siderophores, chitinase activity, NH_3, HCN by *B. subtilis* B38 and *Ps. fluorescens* B103. Data presented in Tables 3a clearly indicated that when *B. subtilis* B38 grew in poly agar medium, the highest inhibition percentage of *F. oxysporum*, *R. solani* and *S. rolfsii* achieved being 80.3%, 79.0% and 71.8%, respectively.

Table 3a. Inhibition percentages of antifungal agents produced by *B. subtilis* B38 in solid media

Cultivation media	*B. subtilis* B38					
	F. oxysporum		*R. solani*		*S. rolfsii*	
	Inhibition (%)	Inhibition by volatile antibiotic (%)	Inhibition (%)	Inhibition by volatile antibiotic (%)	Inhibition (%)	Inhibition by volatile antibiotic (%)
M1	66.7	53.3	71.0	69.5	62.0	55.1
M2	71.1	54.4	70.3	68.9	68.6	52.6
M3	80.3	61.1	79.0	75.8	71.8	61.1
M4	53.3	48.9	55.6	32.2	54.0	22.3
M5	68.9	52.2	59.1	55.6	66.7	55.7

Also, data showed that, the highest inhibition percentage of *F. oxysporum*, *R. solani* and *S. rolfsii* achieved by produced volatile antibiotics were observed when *B. subtilis* B38 grew in poly agar medium being 61.1%, 75.8% and 61.1%, respectively.

Regarding the *Ps. fluorescens* B103, similar trend of results in Table 3b was observed since poly agar medium gave the highest inhibition of *F. oxysporum*, *R. solani* and *S. rolfsii* being 77.9%, 72.4% and 76.1%, respectively. Moreover, *F. oxysporum*, *R. solani* and *S. rolfsii* were inhibited independently by volatile antibiotics produced in poly agar medium with 65.6%, 70.6% and 66.3%, respectively.

Table 3b. Inhibition percentages of antifungal agents produced by *Ps. fluorescens* B103 in solid media

Cultivation media	*Ps. fluorescens* B103					
	F. oxysporum		*R. solani*		*S. rolfsii*	
	Inhibition (%)	Inhibition by volatile antibiotic (%)	Inhibition (%)	Inhibition by volatile antibiotic (%)	Inhibition (%)	Inhibition by volatile antibiotic (%)
M1	71.4	52.0	64.0	60.2	68.9	58.4
M2	70.3	64.4	70.0	63.2	71.1	60.2
M3	77.9	65.6	72.4	70.6	76.1	66.3
M4	59.7	46.7	43.3	26.7	38.0	31.1
M5	66.7	50.6	57.8	47.5	57.8	44.4

M1: Nutrient agar medium (control), **M2:** Tryptic Soy agar medium, **M3:** Poly agar medium
M4: Emerson's agar medium, **M5:** Acetate yeast extract agar medium.

From the above results, it could be mentioned that, poly agar medium was the best medium for production of inhibitive substances for pathogenic fungi by the tested bacteria compared with nutrient agar medium (control). These results may be due to the composition of poly agar medium which has a great effect for improving bacterial strains growth. Since poly medium containing three organic nitrogen sources i.e. beef extract, peptone and yeast extract and supplemented with glycerol as a carbon source. These results are in harmony with those of Rashad *et al* (2012) who reported that poly broth medium has the highest concentrations of C and N which gave the highest yield of bacterial cells.

Concerning the other antifungal activities, obtained data in Table 4a, b revealed that catechol-type siderophores, NH_3 and HCN production as well as chitinase activity by *B. subtilis* B38 and *Ps. fluorescens* B103 were increased on poly medium, while, Emerson's medium showed the lowest production of these antifungal substances. The highest antifungal activities by the tested bacteria may be due to the composition of poly broth medium which has a great effect on improving the protein production by bacterial strains. This result is in agreement with those of Rashad *et al* (2012) who found that the highest significant protein quantities were produced by biocontrol bacterial strains after 24 hours on poly broth medium, whereas culturing of biocontrol bacterial strains in glucose-glutamate-salts-EDTA medium and acetate yeast extract medium produced bioagent compounds lately.

Table 4a. Detection of bioagents factors produced by *B. subtilis* B38

Cultivation Media	*B. subtilis* B38			
	Catecholate-type Siderophores	Chitinase activity (mM NAG amine/ 1ml /1 hr)	NH_3 production	HCN production
M1	+++	6.78	++	++
M2	++	5.30	+++	++
M3	+++	8.50	+++	+++
M4	++	2.65	+	+
M5	++	5.29	++	++

Table 4b. Detection of bioagents factors produced by *Ps. fluorescens* B103

Cultivation Media	*Ps. fluorescens* B103			
	Catecholate-type Siderophores	Chitinase activity (mM NAG amine/ 1ml /1 hr)	NH_3 production	HCN production
M1	+++	4.68	++	+++
M2	+++	4.61	+++	+++
M3	++++	6.60	+++	+++
M4	+	1.50	+	+
M5	++	3.40	++	++

M1: Nutrient medium (control), **M2:** Tryptic Soy medium, **M3:** Poly medium
M4: Emerson's medium, **M5:** Acetate yeast extract medium.

Effect of bacterial cultural filtrate on reduction of fungal biomass

Bacterial cultural filtrates of *B. subtilis* B38 and *Ps. fluorescens* B103 which growing in poly broth medium were evaluated to their effect on suppression of *F. oxysporum, R. solani* and *S. rolfsii* growth after 3 and 7 days (Table 5). The obtained data clearly indicated that all different concentrations of the cultural filtrates gave positive results for suppression of *F. oxysporum, R. solani* and *S. rolfsii* growth after 3 and 7 days. Cultural filtrates with concentration of 10% showed the highest reduction percentage of mycelial growth by the two strains after 3 and 7 days, while culture filtrates with concentration of 2.5% recorded the lowest antagonistic activity.

Moreover, obtained results showed that the maximum reduction percentage of mycelial weight was observed after 3 days by using cultural filtrates compared with the inhibition after 7 days, but the reduction after 7 days may be more realistic because the most fungi needed this period to full growth.

ISSN: 0975-8585

Table 5. Reduction percentage of fungal biomass by *B. subtilis* B38 and *Ps. fluorescens* B103 cultural filtrates

Pathogenic Fungi	Control		Culture filtrate (10%)				Culture filtrate (5%)				Culture filtrate (2.5%)			
	3days	7days	3days		7days		3days		7days		3days		7days	
	D.B. (gL^{-1})	D.B. (gL^{-1})	D.B. (gL^{-1})	R (%)	D.B. (gL^{-1})	R (%)	D.B. (gL^{-1})	R (%)	D.B. (gL^{-1})	R (%)	D.B. (gL^{-1})	R (%)	D.B. (gL^{-1})	R (%)
	***B. subtilis* B38**													
F. oxysporum	11.6	16.2	4.2	63.8	9.0	44.4	6.0	48.3	12.2	24.7	9.8	15.5	15.8	2.5
R. solani	16.6	22.4	9.4	43.4	12.8	42.9	11.0	33.7	17.4	22.3	14.0	15.7	20.2	9.8
S. rolfsii	13.2	20.1	4.4	66.7	12.2	39.3	7.2	45.5	14.6	27.4	8.8	33.3	18.0	10.4
	***Ps. fluorescens* B103**													
F. oxysporum	11.6	16.2	6.2	46.6	10.0	38.3	9.6	17.2	13.6	16.0	10.2	12.1	16.0	1.2
R. solani	16.6	22.4	7.8	53.0	13.0	41.9	10.4	37.3	15.4	31.3	15.8	4.8	20.0	10.7
S. rolfsii	13.2	20.1	5.2	60.6	9.0	55.2	7.2	45.5	11.8	41.2	10.2	22.7	17.0	15.4

- **D.B.:** Dry biomass
- **R (%):** Reduction percentage

Concerning the *B. subtilis* B38 cultural filtrate, results showed that 10 % concentration exhibited the highest reduction percentage of *F. oxysporum* after 7 day being 44.4%. While, the same concentration of *B. subtilis* B38 cultural filtrate gave the lowest reduction percentage of *S. rolfsii* after 7 days being 39.3%. On the other hand, 10% of *Ps. fluorescens* B103 cultural filtrate gave the highest efficient in reduction of mycelial weight of *S. rolfsii* after 7 days being 55.2%. While, the same concentration of *Ps. fluorescens* B103 cultural filtrate gave the lowest reduction percentage of *F. oxysporum* after 7 days being 38.3% (Table 5).

The toxicity of cultural filtrates of these bacteria could be attributed to the production of certain antagonistic metabolites i.e. chitinase, siderophores, ammonia, hydrogen cyanide and volatile antibiotics in the cultural media. These results confirm the previous data which showed the efficacy of *B. subtilis* B38 and *Ps. fluorescens* B103 for production of antagonistic metabolites (Tables 1 and 2). The present findings are corroborated with those of Abou-Aly (2008) who found that the maximum reduction (%) in mycelial weight was observed after 3 and 7 days by using filtrates of *Ps. fluorescens, P. polymyxa* and *B. subtilis* for antagonistic action against *Fusarium oxysporum* f. sp *lycopersici* and *Fusarium solani*. He also mentioned that, the toxicity of cultural filtrates of these bacteria may be attributed to the production of certain toxic metabolites, siderophores and or lytic enzymes.

CONCLUSION

It is clear that, in plant rhizosphere, region there are many bacteria have potentialities for controlling plant pathogenic fungi. Among 116 bacterial isolates, two isolates were exhibited the highest production of antifungal activities against *F. oxysporum, R. solani* and *S. rolfsii in vitro* and identified as *B. subtilis* B38 and *Ps. fluorescens* B103. Finally, it could be recommended that using strains of *B. subtilis* B38 and *Ps. fluorescens* B103 to control of soil-borne pathogenic fungi.

REFERENCES

[1] Abou-Aly HE. Evaluation of some rhizobacteria as potent biological control agents *in vitro*. Anna. Agric. Sc., Moshtohor, 2008;46(4): 81-90.

[2] Ahmad Farah, Ahmad Iqbal and Khan MS. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiol. Rese., 2008;1(63): 173-181.

[3] Albuquerque VV, Terao D and Mariano RLR. Growth promoting and biocontrol of Fusarium wilt in micropropagated plantlets of *Musa* sp. International PGPR workshop , 5-10 Oct.,Calicut, India, 2003;pp. 3-8.
[4] Arnow LE. Colorimetric determination of the components of 3, 4-dihydroxyphenylalanine-tyrosine mixtures. J. Biol. Chem., 1937;118: 531-537.
[5] Atlas RM. Handbook of Media for Environmental Microbiology. (2nd Ed.). CRC press, New York, USA. 2005.
[6] Baligh M, Conway KE and Delgado MA. Production of ammonia by *Pseudomonas cepacia* and *Pseudomonas aeruginosa*: Quantification and effect on host and pathogen. In: S. G. Pandalai (Ed.). *Recent Research Developments in Plant Pathology.* Vol. 1, 19. Trivandrum, Research Signpost, India. 1996.
[7] Barnett HL and Hunter BB. Illustrated Genera of Imperfect Fungi. (4th Ed.). Burgess publishing Company. Minneapolis, Minneosota. 1987.
[8] Blumer C and Haas D. Mechanism, regulation, and ecological role of bacterial cyanide biosynthesis. Archives of Microbiol., 2000;173: 170–177.
[9] Bourgouin C, Larget TI and de-Barjac H. Efficacy of dry powder from *B. sphaericus* RB80, a potent reference preparation for biological titration. J. Invertebrate. Pathol., 1984;44: 146-150.
[10] Cappuccino JC and Sherman N. In Microbiology, A Laboratory Manual, (3rd Ed.). Benjamin, cummings Pub. Co., New York. 1992.
[11] Chaiharn M, Chunhaleuchanon S, Kozo A and Lumyong S. Screening of rhizobacteria for their plant growth promoting activities. J. KMITL Sci. Tech., 2008; 8(1): 18-23.
[12] De La Fuente L, et al. *Pseudomonas fluorescens* UP61 isolated from *birdsfoot trefoil* rhizosphere produces multiple antibiotics extracts and exerts a broad spectrum of biocontrol activity. Euro. J. Plant Pathol., 2004;110: 671–681.
[13] Hariprasad P and Niranjana R. Isolation and characterization of phosphate solubilizing rhizobacteria to improve plant health of tomato. Plant Soil, 2008;316: 13-24.
[14] Liu YF, et al. Bacisubin, an antifungal protein with ribonuclease and hemagglutinating activities from *Bacillus subtilis* strain B-916. Peptides, 2007;28: 553-559.
[15] Lorck H. Production of hydrocyanic acid by bacteria. Physiol. Plant, 1948;1: 142–146.
[16] Meyer JM and Stintzi A. Iron metabolism and siderophore production in *Pseudomonas* and related species. In: E. Montie (Ed.). Pseudomonas, 201-243. Plenum Press, New York. 1998.
[17] Mojica MV, et al. Antagonistic activity of selected strains of *Bacillus thuringiensis* against *Rhizoctonia solani* of chili pepper. Afri. J. Biotech., 2008;7(9): 1271-1276.
[18] Monreal J and Reese ET. The chitinase of *Serratia marcescens*. J. Microbiol., 1969;689 – 696.
[19] Montealegre JR, Reyes R, Perez LM, Herrera R, Silva P and Besoain X. Selection of bioantagonistic bacteria to be used in biological control of *Rhizoctonia solani* in tomato. Electronic J. Biotech., 2003;6: 115-127.
[20] Nelson PE, Toussoun TA and Marasas W FO. *Fusarium* species: An Illustrated Manual of Identification. University Park, Pennsylvania, Pennsylvania State University Press, 1983;pp.193 .
[21] Palleroni NJ. The genus *Pseudomonas*. In: D. J. Brenner; R. Krieg and T. Staley (Eds.). *The Proteobacteria, Bergey's Manual of Systematic Bacteriology*, (2nd Ed.) Vol. (2): 323-379. Springer, New Yourk, USA. 2005.
[22] Phillips AD, Fox TC, King MD, Bhuvaneswari TV and Teuber LR. Microbial products trigger amino acid exudation from plant roots. Plant Physiol., 2004;136: 2887-2894.
[23] Ramyasmruthi S, Pallavi O, Pallavi S, Tilak K and Srividya S. Chitinolytic and secondary metabolite producing *Pseudomonas fluorescens* isolated from solanaceae rhizosphere effective against broad spectrum fungal phytopathogens. Asian J. plant Sci. and Res., 2012;2(1): 16-24.
[24] Rashad Ferial M, Saleh DW, Nasr M and Fathy Hayam M. Identification of mosquito larvicidal bacterial strains isolated from north Sinai in Egypt. AMB Express j., 2012;10: 8-55.
[25] Reva ON, Sorokulova IB and Smirnov VV. Simplified technique for identification of the aerobic spore-forming bacteria by phenotype. Inter. J. Syst. Evolu. Microbiol., 2001;51: 1361-1371.
[26] Ricker A J and Ricker RS. Introduction to Research on Plant Diseases. Jon. S. Swift Co. Inc., St. Lovis, Chicago, New York. 1936.
[27] Sasaki K, Jiaviriyaboonya S and Rogers PL. Enhancement of sporulation and crystal toxin production by corn-steep liquor feeding during intermittent fed-batch culture of *Bacillus sphaericus* 2362. Biotechnol. Letters, 1998;20: 165-168.

[28] Schallmey M, Singh A and Ward OP. Developments in the use of *Bacillus* species for industrial production. Can. J. Microbiol., 2004;50: 1-17.
[29] Weiss FA. Maintenance and preservation of cultures. In: *Manual of microbiological methods*, 99-119. Edited by the Society of American Bacteriologists, McGraw-Hill Book Co., Inc., New York. 1975.
[30] Weller DM and Thomashow LS. Use of rhizobacteria for biocontrol. Curr. Opin. Biotechnol., 1993;4: 306-311.
[31] Zou CS, Mo MH, Gu, YQ, Zhou JP and Zhang KQ. Possible contributions of volatile-producing bacteria to soil fungistasis. Soil Biol. Bioch., 2007; 39: 2371-2379.